The Statistical Analysis of In-Vitro Chromosome Aberration Assay

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1. Introduction

The purpose of in-vitro chromosome aberration assay (ABS) is to determine whether the test compound is a clastogen which induces structural changes in chromosomes. The ABS belongs to the standard three test battery for genotoxicity testing for pharmaceuticals recommended by the fourth International Conference on Harmonisation (1997, Brussel). A full account of biological details of this assay can be found in Galloway et al (1994). The standard design consists of a negative control and at least three positive dose groups. At each dose a sample, say 200, of metaphase cells is examined microscopically and cells exhibiting at least one type of chromosome aberration are identified. It has been well established that the binomial sampling model can be adopted for proportions of cells with chromosome aberration. (Margolin et al, 1986, Richardson et al, 1988.)

Statisticians and toxicologists suggested the evaluation criteria of the dose response pattern of ABS. Margolin et al (1986) suggested to use Cochran-Armitage trend test (CA). Sofuni et al (1990) considered the dose response to be (strong) positive if it had two significant doses out of three dose groups and decided it to be weakly positive if it had only one significant dose and there was a significant trend. Galloway et al’s criterion for a positive response was a clear dose related increase in the cells with structural aberrations in one experiment or a reproducible single positive dose (Galloway et al, 1994).

We will first formulate the above three procedures in terms of Cochran-Armitage trend test and the Dunnett type test and then compare the performance of these three procedures in terms of Monte Carlo simulation study. Then we will develop a software program from the chosen procedure for the toxicologist’s easy use of a statistical procedure.

2. Methods

Sofuni et al (1990) and Galloway et al (1994) didn’t specify statistical methods for their criteria. However, we may apply CA for detecting trend or dose related increase and for detecting a significant increase over the control we may employ Dunnett type test of comparing the control response with each dose group response. Therefore, these
two criteria can be statistically formulated by combining CA and Dunnett type test. We may note that Sofuni's criterion needs significant results from both CA and Dunnett type test, whereas Galloway's requires only one significant result either from CA and repeated Dunnett type tests. We may also employ Dunnett type test to detect two significant positive doses which corresponds to Sofuni's (strong) positive criterion.

In applying Sofuni's criterion we refer \( \alpha_1 \) and \( \beta_1 \) to the comparison-wise significance level in the Dunnett type test and the significance level of CA, respectively. We define \( \alpha_2 \) and \( \beta_2 \), respectively, to represent the comparison-wise significance level in the Dunnett type test and the CA test in applying Galloway's criterion. The first problem is how to choose \((\alpha_i, \beta_i), \ i=1, 2\) so that the family-wise significance level \( (\alpha) \) is less than or equal to 0.05, say.

The determination of \((\alpha_i, \beta_i), \ i=1, 2\) depends on several design parameters such as the number of positive doses, the total number of cells counted at each dose, and the background rate. It is not easy to determine \((\alpha_i, \beta_i)\) analytically, since CA and the Dunnett type test are not independent. We use Monte Carlo simulation to determine \((\alpha_i, \beta_i), \ i=1, 2\) for the standard design of ABS. Once we determine the suitable values for \((\alpha_i, \beta_i), \ i=1, 2\) we can make power comparison among three procedures, i.e., CA, Sofuni’s and Galloway’s procedures.

3. Results

It turns out that for the monotone increasing dose response curves Sofuni’s procedure is the most powerful and then Galloway’s is the next. However, the determination of \( \alpha_1 \) and \( \beta_1 \) for Sofuni’s procedure heavily depends on the design parameters. Galloway’s procedure is less powerful than Sofuni’s, but it is easier to apply, since it is less dependent on the design parameters.

REFERENCES


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